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Development of Breast Cancer Chemopreventive Drugs

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Abstract Breast cancer is the second highest cause of cancer mortality (19%) estimated for U.S. women in 1993 and accounts for the highest proportion of new cancer cases (32%) in this population. The rate of documented cases increased during the early 1970s and again in 1980–87, probably due to early mammographic detection. Increased knowledge of personal risk may also have been a consideration; however, 60% of women diagnosed with breast cancer have no known risk factor(s), such as family history, early age at menarche, late age at menopause, nulliparity, late age at first live birth, socioeconomic status, contraceptive use, postmenopausal estrogen replacement, or high fat intake. To prevent cancer, one strategy undertaken by the NCI is cancer chemoprevention, or intervention with chemical agents at the precancer stage to halt or slow the carcinogenic process.

An objective of the NCI, DCPC is to develop promising cancer chemopreventive chemical agents as drugs for human use. Briefly, the process begins with identification of potential agents (*e.g.*, pharmaceuticals, natural products, minor dietary constituents) from surveillance and analysis of the literature and from *in vitro* prescreen assays. Data on both efficacy (*i.e.*, biological activities that either directly or indirectly indicate inhibition of carcinogenesis) and toxicity are gathered these sources. Various criteria are used to select and prioritize agents for entry into the NCI, DCPC preclinical testing program. The program begins with battery of *in vitro* efficacy screens using both animal and human cells to select agents for further testing; agents positive in these assays are considered for further testing. In the assay used for breast cancer chemoprevention, 7,12-dimethylbenz(*a*)anthracene (DMBA)-induced mouse mammary organ culture, 64 chemicals have inhibited formation of hyperplastic alveolar-like nodules. A panel of organ-specific animal screening assays are then used to assess efficacy *in vivo*. Two assays relevant for breast cancer chemoprevention are inhibition of *N*-methyl-*N*-nitrosourea- and DMBA-induced rat mammary gland carcinogenesis. Of 89 agents tested, 29 have inhibited cancer incidence, multiplicity, or both in at least one of the mammary assays; 21 agents are currently on test. Highly promising agents are then placed in traditional preclinical toxicity tests performed in two species. Finally, the most promising and least toxic agents enter clinical trials. Phase I clinical trials are designed to investigate human dose-related safety and pharmacokinetics of the drug. Phase II trials are small scale, placebo-controlled studies designed to determine chemopreventive efficacy and optimal dosing

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regimens. Three Phase II trials are in progress or in the planning stage investigating tamoxifen citrate or *N*-(4-hydroxyphenyl)retinamide (4-HPR) as single agents; also, both Phase I and Phase II trials evaluating the combination of 4-HPR and tamoxifen are in the planning stage. Phase III trials involve a large target population, with cancer incidence reduction as the endpoint. Tamoxifen citrate is being tested as a breast cancer chemopreventive in high-risk women in a Phase III trial funded by NCI and under the direction of the National Surgical Adjuvant Breast and Bowel Project. Prevention by 4-HPR of a second primary in the contralateral breast of women surgically treated for Stage I/II breast cancer is being evaluated in a Phase III trial in Italy. Finally, the efficacy of β-carotene or vitamin E in decreasing the incidence of breast, lung, and colon cancer is being determined in a Phase III trial involving nurses 45 years of age or older.

Essential to the completion of Phase II clinical trials is the use of populations with defined, measurable biological alterations in tissue occurring prior to malignancy (*i.e.*, intermediate biomarkers) which can serve as surrogate trial endpoints, instead of the more time-consuming and costly endpoint of cancer incidence. Intermediate biomarkers may be of several types, including histological/premalignant lesions, or those based on genetic, biochemical, proliferative, or differentiation-related properties. The only well-established premalignant lesions in the human breast are ductal and lobular carcinoma *in situ* (CIS). In 1993, an estimated 25,000 new cases of CIS will be diagnosed. These lesions are at high risk of progression to invasive cancer and may be amenable to modulation by a chemopreventive agent. In addition, other types of biomarkers could be identified within the lesions. The goal of this workshop is to identify and discuss the best chemopreventive agents and intermediate biomarkers for use as surrogate endpoints in short-term Phase II breast cancer chemoprevention trials, as well as to design protocols for such trials.

Key words: β-carotene, breast cancer, chemoprevention, clinical trials, ductal carcinoma *in situ*, 4-HPR, intermediate biomarkers, lobular carcinoma *in situ*, surrogate endpoints, tamoxifen, vitamin E

THE PROBLEM OF BREAST CANCER

Breast cancer accounts for the highest proportion of new cancer cases (32%) and is the second highest cause of cancer mortality (≈18%) estimated for U.S. women in 1993 [1,2]. Although breast cancer incidence had been increasing steadily by 1% per year since 1940 [3], documented cases increased at a greater rate (4%) during the early 1970s and again in 1980–87. Approximately three-quarters of the rise in the latter years has been attributed to the use of mammography, which led to the detection of early [carcinoma *in situ* (CIS)] or small (<2 cm) invasive tumors [4,5]. Increased knowledge of personal risk may also have contributed to more screening [3]. The reason(s) for the remaining, and steady, 1% rate increase have not been identified. Much attention recently has been focused on the best use of mammography in cancer screening. In February 1993, the National Cancer Institute (NCI) held an International Workshop on Screening for Breast Cancer with the objective of evaluating the status of mammography; the evaluation was based on a critical review of the most recent available clinical trial data [6]. Essentially, the findings of the Workshop support the generally accepted view that mammography is more effective in detecting neoplasia in postmenopausal than in premenopausal breast tissue [7]. The available data demonstrate a significant benefit of screening in women aged 50–69, but not in those aged 40–49. These conclusions have led NCI to draft new guidelines for breast cancer screening calling for women aged 50 and older to be screened with mammography every 1–2 years and women aged 40–49 to be screened only as advised by a health professional taking family history of breast cancer and other risk factors into account.

The factors which confer increased risk for breast cancer can be categorized as age, estrogen exposure (including parity), genetic susceptibility, lifestyle, and histological/previous lesions. Age is the most important single factor. Breast cancer incidence increases rapidly to age 40, and then plateaus to age 50 [8]. After menopause, the rate again increases, but more slowly. For example, the estimated risk that a 30 year old woman will develop breast cancer is 7% of that of a 60 year old woman; 10 years later, the risk has increased to 35% relative to a 60 year old. A

component of this factor is probably length of exposure to estrogen.

The greatest number of determinants is grouped under estrogen exposure. The total length of exposure to endogenous estrogen—resulting from early age at menarche and/or late age at menopause—significantly increases risk, especially for >30 years duration [reviewed in 8]. There is also evidence that early establishment of regular ovulatory cycles further increases the relative risk (RR) by as much as four-fold [9].

Other factors related to estrogen exposure are parity and age at first live birth. It has been suggested that pregnancy increases the extent of tissue differentiation, which is protective against carcinogenesis [10]. In a case-control study, first full-term pregnancy in women between the ages of 30–34 increased the risk of breast cancer 50% over that of women <19 years of age; nulliparity conferred a similar level of risk [11]. First full-term pregnancies after the age of 35 increased the risk even further [reviewed in 8]. Henderson *et al.* [9] have advanced the theory that the number of ovulatory cycles before the first pregnancy determines an individual's lifetime hazard.

Exogenous estrogen may also influence breast cancer development. Meta-analysis of 32 studies on the effect of oral contraceptives suggests that relative risk increases with >10 years of use (RR = 1.46) [8] and with >4 years of use before the first full-term pregnancy (RR = 1.72) [12]. The relationship between breast cancer and postmenopausal estrogen replacement therapy (ERT) is less straightforward [reviewed in 13]. In a meta-analysis of 28 studies, Dupont and Page [14] found a slight increase in risk (7%) in postmenopausal women on ERT; however, the variability in risk estimates between studies was very high. When the postmenopausal estrogen dose, form, or duration was considered, conjugated estrogens below 0.625 mg/day did not increase risk of subsequent breast cancer.

Genetic susceptibility can involve inheritance of either a specific disease or a familial predisposition. An example of the first type is the Li-Fraumeni syndrome, which is characterized by a germline mutation in the p53 gene. Affected pedigrees show a high incidence of six types of cancer; the relative risk for premenopausal breast cancer is estimated to be 17.9 based on the ratio of the observed incidence rate to that expected from the Connecticut Tumor Registry [15,16].

In familial predisposition, the risk is much less, but still significant [reviewed in 8,17]. The relative risk for a woman with first-degree (mother, sister, both) family history of breast cancer ranges from 1.8–5.6 [18]. For example, the cumulative probability for disease in a 30 year old sister of a bilateral breast cancer patient is 55% by the age of 70; in comparison, the probability for the sister of a unilateral breast cancer patient is 8–18% [8,19,20]. Most inherited breast cancer has been attributed to modifications in the BRCA1 gene located on chromosome 17q21 [21]. Easton *et al.* [21,22] have estimated that 60% of families with three or more breast cancer patients and virtually all families with multiple cases of both breast and ovarian cancer carry alterations in BRCA1. Current evidence indicates that BRCA1 is a tumor suppressor gene [21,23]. It has been suggested that changes in BRCA1 may be linked to breast cancer in the general population as well as to inherited susceptibility [21].

The presence of certain histological lesions or previous invasive disease increases the risk for breast cancer. When benign breast diseases (*e.g.*, fibrocystic disease) are categorized by histology, the relative risk for proliferative disease without atypia (≈1.4) is not statistically different from women without proliferative disease [24–26]. In contrast, a diagnosis of atypical hyperplasia increases the relative risk of cancer to 3.7–4.5. Both types of atypical hyperplasia (ductal and lobular) predict carcinoma of the ipsilateral and contralateral breast with equal frequency (Connolly *et al.*, these proceedings); this is also true for lobular carcinoma *in situ* (LCIS, RR = 7–10) [27–30]. The malignant potential of these lesions has not been demonstrated, although they appear to be risk markers. In contrast, ductal carcinoma *in situ* (DCIS) is recognized as a precursor lesion in the breast [30]. The rate of invasive cancer after the initial diagnosis has been reported as 20–50%, usually within the ipsilateral breast [27,31]. Finally, the rate of recurrence of Stage I/II breast cancer after lumpectomy alone is 39% within 8 years, which decreases to 10% with radiation treatment [28].

Lifestyle factors, such as diet, appear to influence the development of breast cancer. High fat intake has been suggested as a risk factor for postmenopausal breast cancer because of a higher incidence of carcinogen-induced mammary carcinomas in rats given high fat (20% corn oil,

w/w, *i.e.*, ≈36% of calories) compared with low fat diets (5% corn oil, *i.e.*, ≈10% of calories) [*e.g.*, 32,33]. Two large prospective cohort studies in the U.S. (Nurse's Study, National Health and Nutrition Examination Survey), however, failed to find an epidemiological association between calorie-adjusted fat intake and breast cancer risk [34–36]. The problem may be that the fat intake in these studies compared levels at >30% of calories, exceeding the highest level in the rat studies. In contrast, international comparisons show a linear relationship between mortality from breast cancer and dietary fat as percent of calories within the range of the rat studies—10–30% [reviewed in 37].

It should be noted that 60–70% of women diagnosed with breast cancer have none of the risk factor(s) discussed above [3,38]. In addition, most of these factors suggest neither strong etiological influences nor straightforward strategies for prevention of breast cancer, except for continued monitoring. Epidemiological evidence suggests that some aspect of environment or lifestyle has an effect because of the international differences in breast cancer rates. The age-adjusted rate in Japan (19/100,000) is one-fifth of that in the US (77–86/100,000) [8]. When Japanese migrate to the US, the incidence of the next generation approaches that of the host country. Obvious modifiable factors such as fat or calorie intake have not been substantiated as primary prevention strategies. A second strategy to prevent cancer—chemoprevention—was undertaken by the NCI about 10 years ago [39,40]. This paper reviews the NCI approach to cancer chemoprevention, the progress to date, and strategies for development of promising breast cancer chemopreventive drugs.

THE SCIENCE OF CANCER CHEMOPREVENTION

Chemoprevention is defined as intervention with chemical agents before malignancy (*e.g.*, invasion across the epithelial basement membrane) to halt or slow the carcinogenic process. It should be emphasized that conceptual differences exist between clinical development of cancer chemopreventive drugs and cancer chemotherapeutic drugs. Chemopreventive drugs are directed at healthy target populations, although these may be populations at increased risk, and the endpoints are reduced cancer incidence or mortality, or increased latency [41]. In contrast, cancer chemotherapeutics are tested in cancer patients, with decreased tumor size and increased disease-free survival as the endpoints. Different levels of toxicity are acceptable in these two patient populations. In the treatment of advanced cancer, severe toxicity is permissible; in

TABLE I. Examples of Intermediate Biomarkers of Breast Carcinogenesis by Class

Histological
Ductal Carcinoma *In Situ* (DCIS)
Lobular Carcinoma *In Situ* (LCIS)
Atypical Hyperplasia, Ductal or Lobular
Proliferation
Nucleolar Organizer Regions, Increased Number/Nucleus
S-Phase Fraction, Increased
TGF-β Promotion (*e.g.*, Angiogenesis, Immune Suppression, Growth Factor Stimulation)
Growth Factor or Receptor Expression (*e.g.*, EGFR)
Ki-67 Antigen Expression, Increased
Differentiation
Myoepithelial Cell Markers, Decreased (*e.g.*, S-100, Keratin 17, Vimentin)
Altered Cytoplasmic Glycoprotein Expression (*e.g.*, Increased Human Milk Fat Globule Glycoprotein)
Altered Cell Surface Antigen Expression (*e.g.*, MAbs GB3, DF3, A-80)
Periductal Lymphocytic (CD4+) Host Reaction
Genetic
DNA Content (*e.g.*, Aneuploidy, DNA Index)
Nuclear Morphometry (*e.g.*, Nuclear Area, Nuclear Perimeter)
Altered Oncogene Expression (*e.g.*, c-*erb*B-2 Overexpression, c-*myc* Amplification)
Altered Tumor Suppressors (*e.g.*, p53 Mutation, BRCA1)
Loss of Heterozygosity (*e.g.*, Chromosomes 17p, 1p, 18q)
Biochemical
Glucose-6-Phosphate Dehydrogenase Activity, Increased
Estradiol $C_{16}\alpha$-Hydroxylation Activity

chemoprevention, only none to minimal acute and chronic toxicity are admissible [42].

For chemopreventive drug development, one of the most difficult aspects is the long period required for many cancers to develop, and consequently, the apparent requirement for long, expensive clinical trials to test the efficacy of chemopreventives. One approach to this problem is the identification of intermediate biomarkers for evaluating clinical efficacy [43, Boone *et al.* these proceedings]. *Intermediate biomarkers* are biological alterations in tissue between initiation and tumor invasion. It is hypothesized that modulation of one or more intermediate biomarkers by a chemopreventive agent(s) would interrupt carcinogenesis. Validation of a biomarker as a *surrogate endpoint* for clinical trials would be obtained when the final endpoint, cancer incidence, is also decreased as a result of this modulation. Evaluation of these surrogate endpoint biomarkers (SEBs) instead of cancer incidence would then allow chemoprevention trials to be of shorter duration, use fewer subjects, and be lower in cost. SEBs may also allow use of serum or small tissue samples to monitor response. Further, they allow determination of effective doses for Phase II trials and rationale for Phase III trials, and may provide basic scientific contributions to understanding the mechanisms of carcinogenesis.

To model the role of intermediate biomarkers in cancer, it is useful to classify them into premalignant/histological, proliferation-related, differentiation-related, genetic, and biochemical groups. This classification scheme has been applied to biomarkers in various tissues such as colon [44], prostate [42], bladder [45], and aerodigestive tract [46]. Table I is a representative listing of potential intermediate biomarkers in the breast classified in this manner.

CHEMOPREVENTIVE DRUG DEVELOPMENT AT THE NCI

The main objective of the Chemoprevention Investigational Studies Branch (CISB) at NCI, Division of Cancer Prevention and Control (DCPC) is to develop promising chemopreventive chemicals drugs. The strategy for this effort has been described in detail previously [40,44]. Briefly, the process begins with the identification of potential chemopreventive agents (*e.g.*, pharmaceuticals, natural products, minor dietary constituents) from surveillance and analysis of the literature [47] and from the NCI, DCPC testing program. Data on both efficacy (*i.e.*, biological activities that either directly or indirectly indicate inhibition of carcinogenesis) and toxicity are gathered from these sources.

In the NCI, DCPC preclinical testing program, a battery of *in vitro* efficacy assays employing human and animal cells is used to select agents for further testing. An assay for efficacy against breast cancer is carcinogen-transformed mouse mammary organ culture. Agents which inhibit 7,12-dimethylbenz(*a*)anthracene (DMBA)-induced formation of hyperplastic alveolar nodules (HAN) during the initiation and/or promotion phases of carcinogenesis are considered for further testing [48,49]. A panel of animal screening and intermediate biomarker assays which are target organ-specific are then used to assess efficacy *in vivo*. Relevant screening assays for this discussion include inhibition of *N*-methyl-*N*-nitrosourea (MNU)- and DMBA-induced rat mammary gland carcinogenesis. Potential intermediate biomarkers—*e.g.*, proliferating cell nuclear antigen (PCNA), glutathione-*S*-transferase π, c-*myc*, p53, and H-*ras* mutations, as well as premalignant lesions—are also being evaluated in the MNU-induced rat model. A gene transfer model of the premalignant lesion, DCIS, in mice is also under consideration [Gould, these proceedings]. As necessary for further development, promising agents are also evaluated in traditional preclinical toxicity tests performed in two species. The scientific rationale for all of the systems used in the NCI, DCPC program has been described previously [40,50].

The most promising and least toxic potential drugs enter the clinical phase of testing [40,41]. Phase I clinical trials are designed to investigate human dose-related safety, pharmacokinetics, and metabolism of the drug. Both Phase II and III clinical trials are designed for the determination of cancer chemopreventive efficacy. Phase II trials are small scale, placebo-controlled studies which focus on dose-optimization and may include modulation of intermediate biomarkers as study endpoints. Phase III trials involve a large target population, with cancer incidence reduction as the endpoint.

The NCI, DCPC drug development effort has been in progress for about 8 years. Approximate-

ly 200 agents are on test in *in vitro* screens; more than 100 agents are on test in animal efficacy screens. There are approximately 20 agents for which reasonable toxicity data are already available or for which NCI is evaluating toxicity. The most advanced of these agents are coming into Phase I and Phase II clinical trials [51,52].

PROMISING CHEMOPREVENTIVE AGENTS FOR BREAST CANCER

Four agents—β-carotene, *N*-(4-hydroxyphenyl)retinamide (4-HPR), tamoxifen citrate, and vitamin E—are being evaluated in NCI, DCPC Phase II or Phase III clinical studies as potential breast cancer chemopreventive drugs. Of these, the retinoid, 4-HPR, and the antiestrogen, tamoxifen, are furthest along in development.

As a retinoid, 4-HPR, a synthetic amide of all-*trans*-retinoic acid, is an antiproliferative and differentiation-inducing agent. It inhibits induction of ornithine decarboxylase (ODC), a critical enzyme in polyamine biosynthesis; polyamine biosynthesis has been implicated in cell proliferation [53,54]. Other activities of 4-HPR are inhibition of prostaglandin synthesis [55] and tyrosine kinase activity, as well as enhancement of immunoglobulin secretion [56]. 4-HPR may also have a very specific antiproliferative effect on terminal end buds in mammary glands [57]. It has been shown to be an effective inhibitor of mammary gland carcinomas *in vivo* in both rat models [*e.g.*, 58,59] and in mouse mammary tumor virus (MMTV)-positive C_3H mice [*e.g.*, 60]; 4-HPR decreased the development of HAN *in vitro* in mouse mammary organ cultures [*e.g.*, 61,62]. The agent is also on test in the battery of intermediate biomarker studies in MNU-induced rat mammary gland carcinogenesis and is proposed for testing in the mouse gene transfer model. 4-HPR is being developed in the clinic because its toxicity appears to be less than that of other efficacious retinoids.

Phase I trials funded by the Italian National Research Council identified a well-tolerated long-term dose of 200 mg 4-HPR/day with a three-day drug holiday/month [63]. This protocol reduces the potential for ophthalmic disturbances associated with retinoids including 4-HPR. These effects, which include impaired night vision and abnormal rod function, are secondary to decreased plasma retinol levels induced by the retinoids. The NCI-sponsored Phase III trial of 4-HPR (Umberto Veronesi, Istituto Nazionale Tumori) in patients surgically treated for Stage I/II breast cancer is assessing prevention of a second primary in the contralateral breast after 5 years under the same dosing protocol [63, Decensi *et al.*, these proceedings]. The study began in March 1987 and recently stopped accruing at 2972 women. Preliminary toxicity evaluations show no changes in clinical chemistry values (hematological, hepatic) or increases in non-breast cancers after 4194 person-years of experience.

Tamoxifen citrate (Nolvadex®) is in both Phase II and Phase III clinical trials. Its pharmacology and potential as a chemopreventive have been reviewed recently [64]. This triphenylethylene-based pharmaceutical has been used previously in the treatment of advanced breast cancer. Tamoxifen competitively blocks the binding of estrogen to its receptor; estrogen is considered to be a promoter of breast carcinogenesis. The agent has additional effects that may be related to chemoprevention such as inhibition of protein kinase C activity, induction of transforming growth factor β, regulation of calcium-dependent events, and modulation of hormone secretion. Depending on species, age, and the endpoint measured, however, tamoxifen may also have estrogenic effects.

Prior to the clinical trials, tamoxifen was not extensively tested in the NCI, DCPC preclinical testing program because of the wealth of published data on inhibition of both chemical- and radiation-induced rat mammary tumors [*e.g.*, 65,66]. The agent inhibited carcinogen-induced rat mammary gland carcinogenesis in NCI, DCPC-funded studies.

The well-known NCI-funded Phase III trial is assessing tamoxifen citrate (10 mg, 2×/day for 5 years) as a breast cancer chemopreventive drug in a population of women >35 years of age with relative risk equivalent to 60 year old women. Risk is calculated from family history, benign biopsy, atypical hyperplasia or previously excised LCIS, nulliparity, late age at first live birth, and/or early onset of menarche. At this point, the trial, under the direction of the National Surgical Adjuvant Breast and Bowel Project (Bernard Fisher, University of Pittsburgh Medical School), has accrued more than half of the target population (16,000).

Tamoxifen citrate is also being evaluated in a NCI, DCPC-funded Phase II trial (John H. Ward, University of Utah/Utah Cancer Registry). Modulation of moderate to severe atypical hyperplasia is being studied in women (n = 120) with previously treated unilateral cancer (≤2 cm)/CIS or who are first degree postmenopausal female relatives of breast cancer patients. Modulation of atypical hyperplasia is being evaluated before and after tamoxifen treatment using fine needle aspiration cytology.

Additional clinical development work is planned for both 4-HPR and tamoxifen citrate. For example, Phase II trials are being designed to evaluate the effects of these agents on putative SEBs in neoplastic breast tissue. The agents will be tested individually and in combination. Combination treatment with the two agents has been shown to enhance the inhibition of carcinogen-induced mammary carcinogenesis in rats [67]. It was also more effective in reducing the induction of subsequent rat mammary carcinomas after removal of the first cancer than either agent alone [68]. A further advantage of the combination is that it may allow lower doses of each agent, thereby reducing the potential for toxicity.

β-carotene (50 mg, every other day) and vitamin E (600 IU, every other day) are on test in separate arms of a Phase III trial. The chemopreventive effect of the agents on the incidence of breast, lung, and colon cancer is being determined in approximately 41,600 female nurses age 45 or older (Julie E. Buring, Brigham and Women's Hospital).

Additional agents are under consideration for future Phase II clinical trials including oltipraz, 2-difluoromethylornithine (DFMO), and dehydroepidandrosterone (DHEA) analog 8354 (fluasterone). Oltipraz is a synthetic dithiolthione structurally related to naturally occurring dithiolthiones found in cruciferous vegetables. This highly lipophilic drug was originally developed by Rhone-Poulenc for the treatment of schistosomiasis. It was also found to increase glutathione levels in rodents in several target organs and to enhance several phase II metabolic enzyme activities in animals, particularly the glutathione-S-transferases, which are involved in carcinogen detoxification [69]. Oltipraz is now regarded as a prototypic phase II enzyme inducer. In the NCI, DCPC preclinical testing program, oltipraz is one of the most widely effective agents tested, yielding positive results in lung, colon, skin, mammary glands, and bladder. In rat mammary glands, the agent inhibited cancers in both MNU- and DMBA-induced rat models. *In vitro*, oltipraz decreased the formation of premalignant lesions in mouse mammary organ cultures.

Based on completed Phase I clinical trials, the maximum tolerated dose (MTD) of oltipraz appears to be <125 mg/day [70]. The dose-limiting toxicity, fingertip pain and nail discoloration, is reversed when treatment is stopped. Further clinical development will depend on identification of a dosing regimen with acceptable toxicity. A trial (Al B. Benson, Northwestern University) in progress is examining the pharmacokinetics and toxicity of lower doses (20–125 mg/day) administered for six months to patients at increased risk for colon or breast cancer, *i.e.*, first degree relatives of breast cancer patients.

DFMO is an irreversible inhibitor of ODC. *In vitro*, it reduces the incidence of HAN. *In vivo*, DFMO is effective against carcinogenesis in many organs, including both MNU- and DMBA-induced rat mammary cancers. Completed Phase I clinical trials have defined a well-tolerated dose (0.5 gm/m^2/day) for extended administration (≥10 months); the most significant adverse effect identified was loss of hearing acuity [71]. Phase II studies in populations at high risk for cervical, bladder, and prostate cancer will continue to define an optimally effective dose regimen without side effects.

DHEA is produced by the adrenal glands and is a normal tissue precursor of estrone and testosterone. In both retrospective and prospective epidemiological studies, decreased circulating levels of DHEA and its metabolites have been associated with increased mammary cancer incidence [72]. The steroid is a potent non-competitive inhibitor of mammalian glucose-6-phosphate dehydrogenase (G6PDH). This inhibition has been proposed as the basis for DHEA's cancer chemopreventive activity; hereditary G6PDH deficiency correlates with decreased cancer incidence. The enzyme pathway including G6PDH is the source of the cofactor, NADPH, which is necessary for many processes including DNA precursor biosynthesis, cytochrome P-450 metabolism, and production of oxygen radicals by neutrophils and macrophages. Thus, the potential chemopreventive mechanisms are decreased activation of some carcinogens, reduced DNA

synthesis (proliferation), and inhibition of oxygen radical damage, and prostaglandin production. The 16α-fluoro derivative, fluasterone, has been shown to be a more effective chemopreventive agent than DHEA, and it lacks the androgenic and liver toxicity of the parent compound [72, Schwartz and Pashko, these proceedings]. In the NCI, DCPC preclinical testing program, it inhibited carcinogenesis in the MNU-induced rat mammary model.

Many more agents with potential as chemopreventive drugs for breast cancer are in earlier stages of development. In the NCI, DCPC preclinical testing program, 29 agents have inhibited cancer incidence, multiplicity, or both in the MNU- or DMBA-induced rat mammary cancer models (Table II), and 64 have inhibited HAN formation in mouse mammary organ culture.

INTERMEDIATE BIOMARKERS WITH POTENTIAL AS SURROGATE TRIAL ENDPOINTS

One of the main thrusts of chemoprevention drug development is to review the current status of early markers and to develop research strategies for identifying and validating intermediate biomarkers for breast cancer, particularly those that are useful as surrogates for cancer incidence reduction in clinical trials. The strategy thus far has been to first identify a well-established histological lesion with significant malignant potential in the organ of interest in both preclinical models and Phase II trials. Page [24,73] has shown the increased cancer risk associated with three categories of proliferative breast disease as follows: proliferative disease without atypia, RR = 1.5–2; ductal or lobular atypical hyperplasia, RR = 4–5; and DCIS or LCIS, RR = 8–10. All of these lesions, when measured by computer-assisted image analysis, may serve as quantifiable SEBs [Boone *et al.*, these proceedings].

For example, aneuploidy, a genetic biomarker (Table I), has been detected by image cytophotometric analysis in 50–71% of DCIS [74,75]. Similar evaluation of atypical hyperplasia found a smaller incidence of aneuploidy (35%); thus, this biomarker appears to be differentially expressed in early and late precancerous lesions [74]. Discrimination between types of DCIS (comedo versus non-comedo) also appears feasible using DNA content/nuclear area and nuclear perimeter [76]. Although other biomarkers such as p53 overexpression [Smith *et al.*, these proceedings], c-*erb*B-2 overexpression [Barnes *et al.*, these proceedings], and loss of myoepithelial cytokeratin 17 [77,78] have been demonstrated in DCIS, they have not been investigated in atypical hyperplasia or normal-appearing tissue in a cancerous breast. These biomarkers may be useful as surrogate trial endpoints; the challenge is to design protocols for Phase II trials which demonstrate modification of these endpoints by chemopreventive agents.

COHORTS FOR PHASE II CLINICAL TRIALS OF BREAST CANCER CHEMOPREVENTIVE AGENTS

An obvious potential cohort from the above discussion is patients with DCIS, which accounts for approximately 70% of breast CIS. The histo-

TABLE II. Chemoprevention Preclinical Testing Program Summary of Progress in *In Vivo* Screens and Efficacy Studies: Agents with Positive Results in Rat Mammary Cancer Models

N-Acetyl-*l*-cysteine	Praziquantel	Progesterone
Aminoglutethimide	Fish Oil	Ro 16-9100
BASF-47851	Fumaric Acid	Ro 19-2968
Carbenoxolone	Glucaric Acid, Calcium Salt	RU 16117
β-Carotene (Injectable)	18β-Glycyrrhetinic Acid	Tamoxifen/Tamoxifen Citrate
Curcumin	4-HPR	Temaroten
DFMO	Ibuprofen	Toremifene
DHEA	Indole-3-carbinol	Vitamin D_3
DHEA Analog 8354	Molybdate	Vitamin E Acetate
Ethylvanillin	Oltipraz	

logical subtypes can be grouped as comedo and non-comedo (papillary/cribriform, cribriform, micropapillary) [Lagios, these proceedings]. Comedo-type DCIS, which consists of a thickened layer of ductal cells surrounding a central area of necrosis and microcalcifications, progresses to invasive carcinoma at a much higher rate than non-comedo DCIS, which does not show necrosis. After local excision of DCIS, the recurrence rate ranges up to 50%; recurrence of non-comedo DCIS is approximately 25–30% within 15 years. One possible study group for short-term evaluation of chemopreventive drugs is patients at the time of diagnosis of DCIS or minimally invasive breast cancer. These patients could be treated with chemopreventive agents until surgical removal of the lesion, a period of 1–2 weeks in many institutions. Effects of chemopreventive agents on DCIS and associated lesions could then be evaluated in the excised breast tissue. This may be a useful cohort for a Phase II trial, since the risk is not related to the original biopsy site. Both breasts are at similar risk for cancer.

According to Gump [these proceedings], LCIS is a risk marker, rather than a precursor lesion. The lifetime risk of invasive breast cancer is approximately 20% [77]. LCIS patients may be a useful cohort for a Phase II trial, since the risk is not related to the original biopsy site [79]. Both breasts are equally at risk for cancer, and biopsy is not usually performed [27–30]. Instead, the patients are monitored by physical examination and mammography. During this "watch and wait" period, chemopreventive intervention would be possible. One problem, however, may be the difficulty in detecting this lesion by noninvasive methods (*i.e.*, mammography).

A final approach suggested by Ruffin *et al.* [these proceedings] is the use of a subset of women at high risk for breast cancer, which may be defined epidemiologically, genetically, or pathologically. Women within this pool with a detectable intermediate biomarker (to be defined) would enter the study. The increased cancer risk attributable to this cohort would decrease the number of subjects required for a given statistical power.

PROGRESS AND PROSPECTS IN CHEMOPREVENTION OF BREAST CANCER

To summarize the progress thus far, two very promising agents for the chemoprevention of breast cancer have been identified (tamoxifen, 4-HPR) and are in clinical trials; a clinical trial involving treatment with a combination of the two agents is also planned. Several additional agents have shown efficacy in animal models and will soon to go into Phase II trials (*e.g.*, fluasterone). Finally, numerous additional agents in early stages of development have demonstrated efficacy in animal models; some of these will enter toxicity testing.

The prospects for breast cancer chemoprevention are excellent based on the large base of potential agents; however, two significant challenges need to be addressed. The first challenge is the selection of optimal cohorts which maximize the probability of determining clinical chemopreventive efficacy without interfering with standard therapy. NCI's efforts will concentrate on those cohorts with a high probability of developing cancer, for example DCIS. The studies may also extend to essentially normal women who are at high risk for breast cancer due to epidemiologic or genetic factors. Especially with this cohort, the development of dependable, minimally invasive sampling and detection methods is required, as well as highly desirable, for effective trials. The second challenge is to identify and evaluate potential intermediate biomarkers for short-term trials. Promising biomarkers, such as aneuploidy, altered nuclear size and shape, and S-phase fraction, are quantifiable and have been detected in neoplastic breast tissue. Carefully designed trials measuring the effects of the most advanced agents, such as tamoxifen, on biomarkers using cytomorphometric and cytophotometric techniques should aid in validation of surrogate trial endpoints.

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